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Review Article

LINC00115 Promote the Invasion and Metastasis of Lung Adenocarcinoma *via* the IL6/JAK1/STAT1 Signaling Pathway

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ABSTRACT

Background: Lung Adenocarcinoma (LUAD) is an important subtype of lung cancer with a high incidence and mortality. LncRNA play a vital role in the occurrence and development of various cancers, including LUAD. LINC00115 was found to be highly expressed in Glioblastoma, breast cancer and colorectal cancer tissues compared with para-carcinoma tissue, and certified to regulate tumor cell proliferation, apoptosis, cell cycle, invasion and migration. However, the molecular mechanism of LINC00115 in lung adenocarcinoma still remains unclear.

Methods: 72 pairs of fresh cancerous and paracancerous tissues were investigated by PCR and RT-qPCR. The diagnostic value of LINC00115 in lung adenocarcinoma was analyzed by ROC curve. Cytological experiment *in vitro* was detected by cell counting kit-8 (CCK-8) experiment, clone formation assay, cell scratch test, flow cytometric apoptosis and cycle assay, and transwell assay (for cell invasion and cell migration). Besides, the proliferation and invasion and metastasis of LINC00115 cells *in vivo* were tested by subcutaneous tumor bearing and tail vein injection in nude mice. Ln CATLAS database and RNA Fluorescence *in Situ* Hybridization (FISH) assay are used to predict subcellular localization of LINC00115. RNA pull-down assay and MS analysis were used to identify LINC00115 binding proteins (RBPs). Names and binding sites of LINC00115 binding proteins were confirmed by cat RAPID and ENCORI database. Binding protein of LINC00115 was detected by Western Blot (WB) assay.

Results: LINC00115 was upregulated in lung adenocarcinoma compared with para-carcinoma tissue. LINC00115 was closely correlated with TNM staging and lymph node metastasis in patients with lung adenocarcinoma, and was negatively correlated with patients' age, gender, tumor size, smoking or not. The Receiver Operating Characteristic (ROC) curve analysis was a high diagnostic value of LINC00115 for patients with lung adenocarcinoma. Nude mice model of pulmonary metastasis *via* tail vein injection was performed to knock-downed LINC00115 had significantly inhibited on the invasion and metastasis of lung adenocarcinoma compared with the control group. LINC00115 was located in the cytoplasm. LINC00115 directly binds to the KHSRP protein. The result of RT-PCR that LINC00115 promoted the invasion and metastasis of lung adenocarcinoma *via* IL6/JAK1/STAT1 signaling pathway.

Conclusions: LINC00115 directly recruits KHSRP protein to promot the invasion and metastasis in lung adenocarcinoma *via* IL6/JAK1/STAT1 signaling pathway. Our findings provided a new biomarker and potential therapeutic targets for clinical diagnosis of lung adenocarcinoma.

Keywords: LINC00115; Lung adenocarcinoma; KHSRP; Invasion and metastasis; IL6/JAK1/STAT1 signaling pathway

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Abbreviation: LUAD-Lung adenocarcinoma

INTRODUCTION

Non-Small-Cell Lung Cancer (NSCLC) constitute approximately 85% of the total LC cases Lung adenocarcinoma (LUAD) is a most common histologic type of non-small cell lung cancer (NSCLC; approximately 60%) [1,2]. LUAD begins to invade and metastasizes early [3]. So far, the underlying molecular mechanisms of LUAD carcinogenesis was not yet been fully understood, and the 5 years survival rate is less than 30% [4,5]. Hence, seeking new molecular targets for LUAD may provide a new train of thought for the treatment of LUAD.

Long non-coding RNAs (lncRNA) are a heterogeneous group of transcripts of more than 200 nucleotides in length, and their dysregulation in a common phenomenon in different cancers, and have emerged as crucial regulators in human cancers [6,7]. Mammalian genomes contain tens of thousands of long non-coding RNA loci, some of which have been found to play an important role in tumor progression. However, it remains challenging to determine the function and mechanism of these lncRNA. A growing body of research shows that lncRNA play a crucial function in the progression of cell cycle, proliferation, migration, apoptosis, invasion and metastasis [8,9]. LncRNA as tumor-suppressor or oncogene have been confirmed to affect the progression of cancer, including lung adenocarcinoma. Thus, lncRNA are not only potential clinical biomarkers for cancer diagnostics and prediction but also possible therapeutic targets [10,11]. LINC00115, a newly discovered lncRNA. Current studies have found that LINC00115 play a crucial role in a variety of malignant tumor. For example, LINC00115 expression is increased in trip-negative breast cancer tissue compared with paracarcinoma tissue, and knock-downed LINC00115 suppresses breast cancer cell migration and invasion. Furthermore, LINC00115 promotes breast cancer metastasis through regulation the expression of miR-7 and KLF4. LINC00115 has been reported to be involved in the poor outcome of patients with breast cancer [12]. LINC00115 is highly expressed in GBM, and LINC00115 expression or co-expression with ZEB1 or ZNF596 are prognostic for clinical GBM survival. LINC00115 was a potential target for GBM treatment [13]. LINC00115 serves as an oncogene in CRC metastasis, and LINC00115/miR-489-3p axis might provide potential therapeutic targets against CRC metastasis [14]. But whether cell function and molecular mechanism of LINC00115 in lung adenocarcinoma is unknown.

LITERATURE REVIEW

LncRNA are characterized by numerous, various function types, modes of action of human physiology and disease. Although a few types of lncRNA have been found to have a clear diagnostic and prognosis role in lung adenocarcinoma, 40% of patients still have primary refractory diseases or higher postoperative recurrence rate. At present, many factors in the pathogenesis of lung adenocarcinoma are still unknown. In this sense, it is still necessary to identify novel molecular and biological candidate biomarkers with diagnostic potential, and continue to conduct complementary experimental studies to further demonstrate regulatory mechanism of lncRNA in the pathogenesis and progression of lung adenocarcinoma. Therefore, in this study, LINC00115 as a specific marker for the diagnosis of lung adenocarcinoma was further studied in an experimental and in-depth way on the basis of previous extensive studies. We hope contribute to the selection of new clinical therapeutic targets and individualized treatment regimens for lung adenocarcinoma.

Patients and clinical specimens

72 fresh tissue samples were collected from patients with LUAD who had undergone surgery at the Huaihe Hospital, Henan University from April 2020 to Oct 2020. The matched adjacent para-carcinoma tissues were collected from distance of more than 5 cm from tumor as control group. The tissues were cut into about 1.5 cm^3 × 1.5 × 1.5 cm^3 after surgery and stored in nitrogen liquid immediately. All tumors were histologically identified as LUAD. All patients did not receive any treatment (including chemotherapy or radiotherapy) and signed informed consent voluntarily. This study was accredited by the Ethics Committee of Henan University. The approval number is HUSOM2020-250.

RNA extraction, reverse transcription and RT-qPCR assay

Total RNAs of LUAD tissue samples and cell lines were extracted using TRIzol reagent (Thermo Fisher Scientific, China). Then, the complementary DNA (cDNA) was synthesized using Prime Script ®RT Reagent Kit with gDNA Eraser (Takara, Henan, Japan) according to manufacturer's instructions. PCR and RT-qPCR was performed using SYBR Green PCR kit (Takara, Henan, Japan) and TB Green Premix Ex Taq II (Takara, Henan, Japan). The relative expression was analyzed using the 2ˆ (-delta, delta, CT) method. And Glyceraldehyde 3-Phosphate Dehydrogenase (GAPDH) was used as an endogenous control for normalization. The sequences of total primers were shown as follows: 1317 bp of L INC00115,F5'-5GACCCGGAAACGGATTATCCGGAGCCA A -3′ R5- TATCTTTGAGCAATTTATTCGTTATGAAC -3; 452 bp of GAPDH, F5- ACCACAGTCCATGCCATCAC -3, R 5′ TCCACCACCCTGTTGCTGTA-3'

The sequences of fragment primers were shown as follows: 187 bp of LINC00115, F5′-AGCGGTGACTGTTGGGTG-3′, R5-CATCCACAGCGAGGCAAT-3′; 131 bp of GAPDH, F5 ATGACAACTTTGGTATCGTGG -3', R 5' AGGGATGAT GTTCTGGAGAG -3'.

After the reaction, CT values of each experimental group and control group were copied from the qPCR instrument (LightCycler480II, USA) to examine the amplification curve and fusion curve.

Cell culture and *Lentivirus* infection

Four human LUAD cell lines (A549, H1975, H1299 and H1437) and normal bronchial epithelium cell line (16HBE) were purchased from Cell Bank of the Chinese Academy of Sciences. A549 and H1299 cells were cultured in RPMI-1640 medium (Gibco, USA) containing 10% fetal bovine serum (FBS; Gibco, USA) and 1% penicillin-streptomycin (penicillin 500 ug/mL and streptomycin 500 μg/ml) double antibody (Gibco, USA). H1975 and H1437 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) (Gibco, USA) containing 10% fetal bovine serum (FBS; Gibco, USA) and 1% penicillin-streptomycin (penicillin 500 ug/mL and streptomycin 500 μg/ml) double antibody (Gibco, USA). 16HBE cells were cultivated with in special medium (Yipu, Wuhan, China). Moreover, all cells were maintained in a humidified atmosphere of 95% O_2 and 5% CO_2 at 37°C.

The *Lentiviruses* LINC00115 and negative controls were obtained from GenePharma (Shanghai, China). Experimental groups in this study comprised LINC00115 overexpression group and LINC00115 silencing group. Culture medium, polybrene (8 μg/mL), and virus solution (MOI=100) were successively added to the cells (total volume 1 mL) following the manufacturer's instructions (GenePharma). After incubation for 48 h, total RNA was extracted, and the infection efficiency was evaluated by RT-qPCR.

Cell Counting Kit-8 (CCK-8) assay

Cell were collected and seeded into the wells of 96-well plates (1000 cells/well). The experimental and the control group were equipped with 3 duplicate wells. PBS was added to other wells around the tested sample to prevent edge effect. At the indicated time (0 h, 24 h, 48 h, 72 h), 10 μl CCK-8 stock solution reagent (Dalian, China)was added to each well for reaction respectively 2 h. Subsequently, the absorbance at 450 nm was measured to evaluate the viability of cells once daily using a microplate reader (InfiniteF50 ELIASA, Thermo, USA).

Colony formation assay

48 h after transfection, the cells were inoculated into 6-well plates. 1000 cells were added to each well, and 3 duplicate wells. The medium was replaced every 3 days-5 days, cultured in complete medium for 2 weeks-3 weeks until obvious colonies were observed. Subsequently, the cells were fixed with 10% formaldehyde (Chuandong chemical industry, Chongqing, China) for 30 min, stained with 1.0% crystal violet (Beyotime, Henan, China) at room temperature overnight, and carefully washed with sterile water. Photographs were taken and the data were processed. The number of visible colonies was counted by ImageJ software.

Flow cytometry cell apoptosis assay

First, cells in good growth state at logarithmic growth stage were taken, and EDTA-free trypsin was used to collect cells, respectively, and inoculated in a six-well plate. After 48 h or 72 h, Annexin V-FITC/PI apoptosis kit (Liankebio, Hangzhou, China) was operated according to the manufacturer's instructions. Centrifugal washing with precooled PBS, 6 × 10^5 cells (including cells in the culture supernatant) were harvested. Dilute 5 × binding buffer with double steam water into 1 × working solution. Then take 500 μl 1 × binding buffer to resuspend the cells. Add a nnexin V-FITC 5 μl and annexin PI 10 μl to each tube. After sufficient vorticity and mixing well. Cells were incubated at room temperature in dark for 5 minutes. Finally, Beckman Coulter CytoFLEX flow cytometry (Beckman Coulter, Inc, USA) was used for detection within 1 hour. CytExpert software captures and analyzes experimental data.

Flow cytometer cell cycle assay

Stable transfection of cells in good growth state at logarithmic growth stage were taken, and trypsin digestion counted about 5 × 10^5 cells, respectively, and inoculated in a six-well plate. After 24 h or 48 h, the cells were digested with trypsin without EDTA, and cells were collected. The cells were centrifuged at 1500 rpm for 5 min at room temperature. The supernatant was removed, and then washed with pre-chilled PBS. The cell concentration was adjusted to 1 × 10^6/ml, and 1 ml single-cell suspension was taken. The prepared single-cell suspension was centrifuged, the supernatant was removed, 70% pre-cooled ethanol 500 ul was added into the cells for fixation, and the solution was kept overnight at 4°C. Centrifuge and discard supernatant. 1000 ul RNase a solution was added to the cell precipitation, the cells were resuspended and bathed at 37°C for 30 min. Then 400 μl PI staining solution was added to mix well and incubated at 4°Cin dark for 30 min. Finally, the cells were analyzed using a Beckman Coulter CytoFLEX flow cytometer (Beckman Coulter; USA), and the experimental data were processed by cell cycle analysis software (FlowJo7.6).

Wound healing assay

Stable transfections of cells in good growth state at logarithmic growth stage were taken, and trypsin digestion counted about 6 × 10^5 cells, and inoculated in a six-well plate. After 24 h or 48 h, cells were then washed twice with PBS. The cells monolayers were wounded by scratching with plastic 200 μl micropipette tips, and then put in 37°C, 5% CO_2 incubator culture. Images of the different stages of wound healing were photographed *via* microscopy at 0 h, 12 h, 24 h, 48 h, 72 h. Image J software was used to analysis.

Transwell invasion and migration assay

Cells in logarithmic growth stage were digested and counted about 1×10^6 cells respectively and inoculated on six-hole plate. After 24 h or 48 h, the cells were digested and collected by trypsin. Use serum-free RPMI-1640 medium and DMEM medium to resuspend cells, count cell with a cell counter plate, dilute cell concentration to 6 × 10^5/ml. Matrigel (BD

Biosciences, USA) (200 μg/ml) of 100 ul in the center bottom of the transwell plate upper chamber was placed in 37°C 5% CO_2 incubator for 2 h, and then the upper liquid medium (at which point the Matrigel matrix sinks to the bottom to form a thin layer) was carefully discarded. The upper chamber was 200 μl FBS-free medium, lower chamber was 700 ul complete medium, 37°C, 5% CO_2 incubator culture 24 h or 48 h. The cells were fixed with methanol solution (Upper chamber 200 μl, Lower chamber 700 μl) for 30 min. The chamber was placed in the PBS and washed the methanol slowly. The invasive cells were stained with 0.1% crystal violet dye indoor temperature for 4 h-6 h. The invasive cells were observed and were took photos. 5 field's cells under high magnification (400 x) were randomly counted. The steps of the migration assay are all the same as invasion assay, except that matrix glue is not required to chamber.

BALB/C nude mice animal experiment

Male BALB/c nude mice (ages 4 weeks-5 weeks and 16 g-20 g) were obtained from Vital River Laboratory Animal Technology Co. Ltd (Beijing, China) and randomly divided into two groups (n=10 per/group). For the *in vivo* tumorigenicity assay, H1437 cells that had been stably transfected with sh-LINC00115 or sh-NC were implanted subcutaneously into the scapula of nude mice to form LUAD tumor model. The injection dose was 5×10^6/ml subcutaneously on each side per mouse. Generally, the tumor can be seen subcutaneously 5 days after injection. The tumor volume was calculated with calipers every 2 days using the following formula: length (L) × width $(W)^2/2$. After 22 days, nude mice were sacrificed, and the tumors were removed and weighed. Part of the tissue was fixed with paraformaldehyde for HE and Ki67 dye assay. The rest were preserved at 80°C for RT-qPCR or western blot assay. For the *in vivo* tumor invasion assay, 1×10^6/ml cells were injected intravenously into the tail vein of nude mice (n=10/group). The injected cells were respectively stable transfection knock down or overexpression and their control cells. Weigh yourself every 3 days until 49 days-63 days. Experimented mice were to proceed with general anesthesia and put to death by intracardiac injection. All animal research procedures were approved by the animal experimental ethics committee of medical college, Henan university.

RNA Fluorescence *In Situ* Hybridization (FISH)

FISH assay was performed in H1437 cells according to the manufacturer's instructions of FISH kit (GenePharma, Shanghai, China). FISH Cy3-labeled LINC00115 Probes was designed and synthesized by GenePharma's (Shanghai, China) online design software. Briefly, Cells were seeded into 24-well plates at a density of 2×10^4 cells/well. Put the properly sized cover glass slides into the wells in advance, and culture them overnight in the incubator before sticking to the wall. The medium was absorbed and discarded, and washed twice with PBS, 5 min each. Cell were fixed in 4% formaldehyde for 15 min and 4% paraformaldehyde was absorbed and discarded, and 0.1% Buffer A was added to each well to treat cells at room temperature for 15 min. 0.1% Buffer A was absorbed and washed twice with PBS, 5 min each. Absorption and discard PBS, add 2 × Buffer C to each well, and place in incubator at 37°C for 30 min. Absorb and discard 2 × Buffer C, then add 200 μl probe mixture to each well, take protection from light and place in 37°C incubator overnight for hybridization. After hybridization, the slide was washed, dehydrated, and add 200 μl diluted DAPI working solution to each well, and stain for 20 min in dark. Absorb and discard DAPI working liquid, wash with PBS twice, 5 min each time. Glycerin or anti-quenching agent was added to a clean slide, and the cells were covered face down on the slide. The slide was sealed with glue and observed under a fluorescence microscope. The images were captured using a confocal microscope. Biology replicates were counted three times.

RNA pull-down and Mass Spectrometry (MS)

Biotin-labeled LINC00115 and negative control (Biotin-NC) were synthesized from GenePharma (Shanghai, China). H1437 cells were transfected with the above probes and were incubated for 24 h. After that, cells were lysed and Magnetic bead pretreatment and magnetic bead enrichment of RNA. RNA protein binding and protein elution. Finally, the silver dyeing experiment was done. Elution products of RNA pull-down were analyzed by mass spectrometry and sequenced. Briefly, Number the silver dyeing tape, decolorize, reduce alkylation, and then carry out enzymatic hydrolysis. Subsequently, the enzyme-hydrolyzed polypeptides were extracted, desalted, dried in vacuum, and 1-2 ug of the samples were put into the machine. Mass Spectrometry Data Analysis Software: Proteome Discoverer 2.4 (Sequest HT), Thermo Scientific, USA. Finally, WB was used to verify the results of MS analysis.

Western blot (WB) analysis

Total proteins were obtained using RIPA lysis buffer (Beyotime Shanghai, China). After incubating for 30 min on ice, the above cell lysates were centrifuged at 12,000 rpm for 5 min at low temperature. Next, the concentration of protein in different cell lysates was quantified using the Pierce™ BCA protein assay kit (Thermo Scientific, Henan, USA). The proteins were denatured and separated to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on 8% denaturing gel and transferred onto Polyvinylidene Fluoride (PVDF) membranes (Millipore, Zhengzhou, China). Then, the membranes were blocked with 5% nonfat milk in tris buffered saline (TBS)-Tween 20 (0.05%, v/v) for 1 h at room temperature and used to wash by TBST. Then, the membranes were incubated with diluted primary antibodies (KHSRP; 1:1000, Yiqiao, Beijing, China), and GAPDH (1:500, Yiqiao, Beijing, China) overnight at 4°C. Subsequently, respective secondary antibody (1:1000, Yiqiao, Beijing, China) conjugated with horseradish peroxidase was incubated for 2 h at room temperature. Ultimately, the membranes were added with enhanced chemiluminescence developing solution (Beyotime, Henan, China) to visualize the protein blots. Then, the protein bands were quantified using E-Gel Imager (Invitrogen).

Statistical analysis

All statistical analyses were performed using the GraphPad Prism 8.0 software (GraphPad Software Inc, CA, and USA). The statistical methods used in this paper are: t-test, chi-square test, ordinary one-way ANOVA, etc. Each experiment was repeated three times. A p-value <0.05 was considered statistically significant.

RESULTS

LINC00115 is significantly up-regulation in LUAD tissues compared with para-carcinoma tissues, which is positively associated with TNM staging and lymph node metastasis.

Through the ln CAR database analysis of LINC00115, we performed it is located on chromosome 1(chr1:826206-827522), and there are two transcripts and one exon. In ln CAR database and 72 fresh lung adenocarcinoma and para-cancerous tissue samples, LINC00115 expression was markedly increased in LUAD tissues compared with adjacent para-carcinoma. Meanwhile, 72 cases of LUAD and para-carcinoma tissues were divided into 36 high-expression group and 36 low-expression group according to the median 1.2227 expression level of LINC00115 $2^{-\Delta\Delta Ct}$. The correlations between LINC00115 expression and the clinical pathological features of lung adenocarcinoma patients suggested that high expression of LINC00115 was positively correlated to lymphatic metastasis and TNM stages of lung adenocarcinoma patients (both P<0.05, Table 1). High expression of LINC00115 have no connection with the sex, age, smoking history, tumor size, and differentiated degree of LUAD patients (all P>0.05, Table 1). Univariate analysis showed that the higher TNM stage of LINC00115, the higher the expression of LINC00115. The higher expression of LINC00115 was in the group with late lymph node metastasis than in the group with early lymph node metastasis. Receiver Operating Characteristic (ROC) curve analyses showed that the Area Under Curve (AUC) of LINC00115 increased with high TNM stage and lymph node metastasis. These suggest that LINC00115 may serve as a novel biomarkers and potential therapeutic targets for LUAD.

LINC00115 promotes cell invasion and metastasis of LUAD *in vitro*

To explore the biological functions of LINC00115 in LUAD cell lines, we examined the expression of LINC00115 in different LUAD cell lines by performing PCR and RT-qPCR assays. The result showed that LINC00115 was significantly increased in H1975 and H1437 and obviously decreased in A549 and H1299. H1437 and A549 cell lines would be selected for subsequent cell function test. Firstly, knock-downed LINC00115 with *Lentivirus* stable transfected into H1437 and over-expressed LINC00115 with *Lentivirus* stable transfected into A549. The results showed that expression of LINC00115 were remarkably inhibited in H1437 and remarkably promoted in A549. The above results indicated that cell lines of LUAD have been successfully stable transfected and the next functional experiments could be carried out. CCK-8 assay revealed that knock-down of LINC00115 could not markedly suppress the cell proliferation of H1437 and overexpression of LINC00115 could not markedly promote the cell proliferation of A549. Similarly, the colony formation assays have also shown that LINC00115 both knockdown and over-expression could not significantly reduce cell colonies compared with siRNA-NC or Vector. (Cell apoptosis rates of LUAD cells were detected using flow cytometry. Compared with those in the control group, LINC00115 knock-down increased cell apoptotic rate of H1437, including early and lately and total cell apoptosis.

Similar results were observed in A549, LINC00115 over-expression decreased apoptotic rate of A549, including early and lately and total cell apoptosis compared to control group.

Moreover, our flow cytometry results showed that knock-down and over-expression of LINC00115 had not obviously promoted and inhibit G0/G1 and S process. Furthermore, scratch wound assay results determined that LINC00115 knock-down inhibited the ability of H1437 repairing wound compared to the control group. In contrast, LINCOO115 over-expression promoted the ability of A549 repairing wound compared to the control group. Meanwhile, Transwell assay results revealed that LINC00115 knock-down restrained the invasion and migration of LUAD cells and LINC00115 overexpression promoted the invasion and metastasis of LUAD cells when compared with those in control group. Therefore, these above results suggested that LINC00115 might play an oncogenic role in LUAD cell lines.

LINC00115 promotes cell invasion and metastasis of LUAD *in vivo*

To further verify the effect of LINC00115 on LUAD tumorigenicity and metastasis *in vivo*, nude mouse xenograft tumor model and pulmonary metastasis model *via* injecting into the tail vein were constructed. Nude mouse xenograft tumor model assay, each nude mouse was injected 5×10^6/200 μl under the scapula. Observation was performed every 2 days up to 28 days. Growth rate of tumor volume in the LINC00115 knock-down group was not significantly inhibited compared with the control group. Besides, we also discovered that the tumor weight was not markedly decreased in the LINC00115 knock-down group compared with the control group. HE staining showed no difference in tumor cell proliferation rate between the SH-NC group and the SH-LINC00115 group. Ki-67 staining showed no significant difference in tumor cell proliferation index between sh-NC group and SH-LINC00115 group. Moreover, Nude mice xenograft transfer models assay, each nude mouse was injected 1×10^6/200 μl through the caudal vein, every 2 days until 63 days. Our results showed that both knock-down and overexpression LINC00115 had significant difference in lung metastasis compared with the normal group. The above experimental results of nude mice *in vivo* were consistent with the results of cell function experiments *in vitro*. These data again confirmed that LINC00115 played an active function of invasion and metastasis in lung adenocarcinoma.

LINC00115 mainly locates in cytoplasm and directly binds to KHSRP protein in LUAD

The subcellular location of lncRNA plays an important role in helping us interpret and understand the mechanisms of lncRNA function. The subcellular distribution of LINC00115 transcripts were predominantly located in the cytoplasm of H1437 cells, which indicated that LINC00115 mainly played a regulatory role in the posttranscriptional level in LUAD. We verified the binding possibility of LINC00115 and KHSRP protein *via* silver staining technique and WB assay. Mass spectrometry results showed that LINC00115 was bound to KHSRP protein. The possible binding proteins and binding sites of LINC00115 were analyzed by CATRAPID and ENCORI databases. WB assay verified LINC00115 binding to KHSRP protein.

LINC00115 promotes the malignant progression of LUAD *via* the IL6/JAK1/STAT1 signaling pathway

The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway and Gene Ontology (GO) enrichment analysis were performed using the online biological information annotation database DAVID, P<0.001 and False Discovery Rate (FDR)<0.001 were used as cut-off criteria. The Protein-Protein Interaction (PPI) network of the binding protein of LINC00115 was predicted by the STRING database. We verified the expression of sh-LINC00115 group and sh-control group in the IL family members, IFN family members, JAK family members and STAT family members by RT-qPCR. Then, we verified the expression of sh-LINC00115 group and sh-control group in KHSRP by RT-qPCR. The results showed that LINC00115 was consistent over IL6, JAK1 and STAT1, with significant differences.

KHSRP promotes the malignant progression of LUAD *via* the JAK1/STAT1 signaling pathway WB results of KHSRP protein regulates invasion and metastasis of lung adenocarcinoma through JAK1/STAT1 signaling pathway. Altogether, these results suggest that LINC00115 interacts directly with KHSRP, acts as an oncogenic genes to promote the malignant phenotype of LUAD through the JAK1/STAT1 signaling pathway.

DISCUSSION

LUAD is typically featured by a low 5-years survival rate, hence there is an essential need to investigate new diagnostic biomarkers in LUAD progression [15]. LncRNA have served as either oncogenic factors or tumor suppressors, then played an important role in tumor occurrence and development, and have been involved in carcinogenesis, tumor invasion and metastasis. Recently, increasingly lncRNA are identified as potential key regulators in tumorigenesis and cancer progression [16]. More and more of lncRNA have been confirmed to serve critical roles in LUAD cell proliferation, apoptosis, cell cycle, migration and invasion [17]. LINC00115 has been found to play an important regulatory role in the occurrence and development of breast cancer, colorectal cancer, and glioma. Our results showed that LINC00115 expression was significantly increased in LUAD tissues compared with adjacent para-cancerous tissues. We also observed that upregulation of LINC00115 was strongly correlated with advanced TNM stage, lymphatic metastasis of LUAD patients (P<0.05), or ROC=0.7541, above all of these suggesting that LINC00115 could be a potential diagnostic marker of LUAD. LUAD has a high mortality rate and poor prognosis, so appropriate screening and early diagnosis is an important means to improve the survival rate [18]. Novel diagnostic lncRNA have indeed played an important role in pathogenesis of malignant tumors by acting as either potential oncogenes or tumor suppressors, reducing the mortality and improving the prognosis quality of life [19]. In order to improve the diagnostic performance, more comprehensive studies are needed to continuously supplement novel diagnostic lncRNA and improve the diagnostic value of lncRNA in LUAD. The quantity of lncRNA in the diagnosis of lung adenocarcinoma is gradually increasing, and researchers have proposed the specific clinical applications of these lncRNA, bringing new light to our comprehensive understanding of the signaling pathways in lung adenocarcinoma [20,21]. Screening and identification of biomolecular markers that can accurately predict LUAD, and gradually trying to develop a more accurate personalized targeted therapy through the molecular mechanism of LUAD, then improve quality of life and prolong survival time of patients[22,23]. In particularly, it is of a great practical significance and theoretical value to carry out further experimental research on the pathogenesis and progression of lung adenocarcinoma and explore more effective biological treatment methods at the molecular level.

Our Cell function experiment *invitro* disclosed that knockdown of LINC00115 inhibited invasion and metastasis of LUAD and promoted apoptosis LUAD. Meanwhile, overexpression of LINC00115 promoted invasion and metastasis of LUAD and inhibited apoptosis LUAD. The results identified that the important biological function and regulatory role of LINC00115 in LUAD and added a new member for the development of lncRNA. The experimental results of lung metastasis model in nude mice by caudal vein injection showed that either knockdown or overexpression of LINC00115 *invivo* also had significantly affected the invasion and metastasis of LUAD. These findings indicated that LINC00115 was associated with the progression of LUAD, but the exact molecular regulatory mechanism was needed to be illuminated. Currently, a growing of lncRNAs have been identified as an oncogene or as a tumor-suppressor gene in various cancer, including almost all common cancers such as breast cancer, lung cancer, pancreatic cancer, liver cancer, colon cancer, stomach cancer and ovarian cancer [24,25]. Moreover, many of the same lncRNA have been reported to have important regulatory functions in a variety of biological processes [26-28]. Current studies have shown that lncRNA were closely associated with six basic characteristics of cancer (unlimited proliferation, resistance to cell death, induction of angiogenesis, cell immortality, enhanced invasion and metastasis, and escaped from growth inhibition) [29]. Therefore, lncRNA play a huge biological regulatory role in the tumorigenesis, and progression of various cancers. Meng, et al. found that lncRNA has emerged as pivotal regulatory molecules in biological processes, which can affect many aspects such as tumor proliferation, cell apoptosis, signaling pathway, tumor invasion and metastasis. Even Liu, et al. [30] believed that lncRNA might be the core regulatory gene of

the pathophysiology and molecular biological mechanism of malignant tumor cell invasiveness and metastatic capacity, and that tumor cell invasiveness and metastatic capacity were closely related malignant tendency. Studies have shown that lncRNA participated in regulating a series of complex biological processes such as cell proliferation, invasion, metastasis, apoptosis, cell cycle and drug resistance of lung adenocarcinoma cells [31,32]. LncRNA also participated in regulating a great variety of molecular mechanisms such as targeting protein-coding genes, mediating chromatin remodeling and histone modification, interfering with mRNA splicing and modulating cytoplasmic-nuclear localization of proteins [33]. In conclusion, lncRNA played a vital biological regulatory role in the era of personalized therapy for a variety of cancer patients including LUAD, and lncRNA also been unique in diagnosis and prognosis evaluation, and had a broad prospect regarded as novel tumor molecular markers.

The subcellular localization of lncRNA to tumor regulatory mechanisms was attractive research areas. The subcellular localization of lncRNA in the nucleus or cytoplasm was closely related to their unique molecular function. Different subcellular loci had different regulatory mechanisms of action. LncRNA located in the nucleus were mainly involved in epigenetic and transcriptional level regulation, including histone modifications, DNA methylation, chromatin remodeling, and transcription factors [34-37]. While lncRNA located in the cytoplasm were mainly involved in post-transcriptional and translational level regulation, including interaction with proteins in the cytoplasm, mRNA metabolism, and as competitive endogenous RNA (ceRNA) interacts with microRNA [40]. In our study, LINC00115 was identified to be localized in the cytoplasm. The biological function of LINC00115 had been demonstrated in many cancers, and we have also discovered that it exerted oncogenic functions in LUAD, which was consistent with its expression in other cancers. LncRNA-protein interaction regulates protein activity in transcriptional level activation and silencing. There were several crucial types of functional lncRNA which participate in some famous signaling pathway, such as: Wnt/βsignaling pathway, MARK signaling pathway, STAT signaling pathway and so on. Therefore, we confirmed that LINC00115 might participate in regulating IL6/JAK1/STAT1 signaling pathway in LUAD. Numerous studies collectively indicated intricate activation or inhibition between lncRNA and JAK/STAT signaling pathway [41,42]. JAK/STAT frequently exerts its properties as an oncogenic signal in human malignant tumor [43,44]. STATs were crucial transcription factor activated by Janus Associated Kinase (JAK) under the stimulation of cytokines such as interleukin, interferon, tumor necrosis factor, and growth factor, and then entered the nucleus to mediate the transcription of downstream target genes. JAK family might mediate signal transduction from cytokine receptors by phosphorylation and activation of intracellular signaling pathways and transcription factors. STAT family as transcription factors, regulated multiple oncogenic signaling pathways [45]. Accumulating studies have revealed that JAK/STAT acted as a key regulator of cellular growth, adhesion, metabolism, migration and invasion *in vivo* carcinogenesis and tumor development, including LUAD. The phosphorylation of JAK is the key step for their activation in regulating various tumors signaling pathway. Once phosphorylation, JAK could activate and phosphorylate its downstream target STAT to cause a cascade reaction. Stat family members were phosphorylated and translocated into the nucleus where they regulated gene transcription. Activated JAKs not only can stimulate downstream specific STAT family members, but also can interact with other STAT family members to form a complex regulatory network. In our conclusion, LINC00115 promoted invasion and metastasis in LUAD *via* IL6/JAK1/STAT1 signaling pathway. The result of above was predicted and verified by bioinformatics analysis and experimental verification method.

In recent study, LINC00115 was shown to regulate various biological processes by activating or inhibiting JAK/STAT signaling pathway in cancers, such as breast cancer, colorectal cancer, and glioma. Interestingly, LINC00115 was found that activated by IL6, then phosphorylated JAK2, and STAT3, stimulated KHSRP protein entering the nucleus, suggesting that LINC00115 positively promoted invasion and metastasis *via* IL6/JAK1/STAT1 signaling pathway. LINC00115 might mediate invasion and metastasis of LUAD *via* IL6/JAK1/STAT1 signaling pathway implicated that it might serve as a novel molecular marker for the diagnosis of LUAD. The elucidation of the molecular mechanism in lung adenocarcinoma also provided valuable references for studying the role of LINC00115 in other cancers. Regrettably, our verification of the LINC00115 only remained at the cell function level *inwitro* and in nude mice level *invivo*. Therefore, subsequent test will further verify the upstream and downstream regulation mechanism of LINC00115/IL6/JAK1/STAT1 axis at different levels. Molecular mechanism of signaling pathways might play a significant role in the regulation of tumor genesis and progression. Consequently, an in-depth study of the IL6/JAK1/STAT1 signaling pathway is helpful to further deepen the understanding of regulating role of LINC00115 in LUAG and provide a new idea for the clinical individualized treatment options of LUAD.

CONCLUSION

In our study, LINC00115 was a tumor-promoter in LUAD. LINC00115 was closely correlated with TNM stage and lymph node metastasis of LUAD patients (all $P<0.05$). LINC00115 can be directly bound to KHSRP protein. LINC00115 may novel potential diagnosis marker for LUAD. We will further increase the tissue sample size in the future to demonstrate again that LINC00115 is indeed up-regulated in lung adenocarcinoma tissues.

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AUTHOR' CONTRIBUTIONS

Li, Li. Lin lin, Fan. Designed the experiments and wrote draft of the manuscript; Hui fang, Cai. Hui qin, Song. Acquired and analyze data; Hai tao, Wei. Li,Li. made critical revision of the

manuscript for intellectual content. All authors provided critical feedback and helped shape the research, analysis, and manuscript.

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AVAILABILITY OF DATA AND MATERIALS

Data availability statement: All data generated or analyzed during this study are included in this article. The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This project was approved by the Ethical Committee on Scientific Research of Henan University (ECSRHU).

CONSENT FOR PUBLICATION

All human tissue samples were obtained with written informed consent from all subjects.

COMPETING INTERESTS

The authors declare that there is no conflict of interests.

REFERENCES

1. Gong H, Li YW, Yuan Y, Li WT, Zhang HB, Zhang ZH, et al. EZH2 inhibitors reverse resistance to Gefitinib in primary EGFR wild-type lung cancer cells. Bmc Cancer. 2020;20(1).
2. Jin-qiong L. Survey of factors influencing women's selection of the delivery method of their second child in Shanxi Province, China. Pak J Med Sci. 2021;37(3):846-850.
3. Stevens LE, Zhao M, Liu Z, Nguyen DJCR. A novel molecular subset of metastatic lung adenocarcinoma is defined by the function of the proteoglycan receptor HMMR. 2015;75(15 Supplement): 2269-2269.
4. Jing S, Sheng Y, Tong L, Wu W, Xu S, Yin L, et al. Molecular characterization of lung adenocarcinoma: A potential four–long noncoding RNA prognostic signature. J Cell Biochem. 2019;120(1): 705-714.
5. Wang J, Su Z, Lu S, Fu W, Liu Z, Jiang X, et al. LncRNA HOXA-AS2 and its molecular mechanisms in human cancer. Clin Chim Acta. 2018;485:229-233.
6. Xing C, Sun SG, Yue ZQ, Bai FJB. Role of lncRNA LUCAT1 in cancer. Biomed Pharmacother. 2021;134:111158.
7. Sun J, Guo Y, Bie B, Zhu M, Li ZJJoCB: Silencing of long noncoding RNA HOXD-AS1 inhibits proliferation, cell cycle progression, migration and invasion of hepatocellular carcinoma cells through MEK/ERK pathway. J Cell Biochem. 2020;121(1):443-457.
8. Barton M, Santucci-Pereira J, Su Y, Russo JJCR. Abstract P1-08-06: BC200 lncRNA is involved in the progression of triple negative breast cancer. 2017;77(4 Supplement):1-08-06-01-08-06.
9. Mouraviev V, Lee B, Patel V, Albala D, Johansen T, Partin A, et al. Clinical prospects of long noncoding RNAs as novel biomarkers and therapeutic targets in prostate cancer. Prostate Cancer Prostatic Dis. 2016;19(1):14-20.
10. Bhardwaj V, Tan YQ, Wu MM, Ma L, Pandey VJCL. Long non-coding RNAs in recurrent ovarian cancer: Theranostic perspectives. Cancer Lett. 2021;502:97-107.
11. Yuan C, Luo X, Duan S, Guo LJFOB. Long noncoding RNA LINC00115 promotes breast cancer metastasis by inhibiting miR-7. FEBS Open Bio. 2020;10(7):1230-1237.
12. Tong JF, Ma XD, Yu HL, Yang JH. SNHG15: a promising cancer-related long noncoding RNA. Cancer Manag Res 2019;11:5961-5969.
13. Feng W, Li B, Wang J, Zhang H, Zhuang J. Long non-coding RNA LINC00115 contributes to the progression of colorectal cancer by targeting miR-489-3p *via* PI3K/AKT/mTOR pathway. Front Genet. 2020;11:567630.
14. Liu H, Qin Y, Zhou N, Ma D, Wang YJCD. ZNF280A promotes lung adenocarcinoma development by regulating the expression of EIF3C. Cell Death Dis. 2021;12(1):39.
15. Liu Z, Mo H, Sun L, Wang L, Yang NJCS. LncRNA PICSAR/miR8/EIF6 axis regulates tumorigenesis of hepatocellular carcinoma by activating PI3K/AKT/mTOR signaling pathway. Cancer Sc. 2020;111(11):4118-4128.
16. Wang AH, Fan JW. LncRNA PCAT-1 regulated cell proliferation, invasion, migration and apoptosis in colorectal cancer through targeting miR-149-5p. Eur Rev Med Pharmacol Sci. 2019; 23(19):8310-8320.
17. Fujimoto M, Yoshizawa A, Sumiyoshi S, Sonobe M, Menju T, Hirata M, et al. Adipophilin expression in lung adenocarcinoma is associated with apocrine-like features and poor clinical prognosis: An immunohistochemical study of 328 cases. Histopathology. 2017;70(2):232-241.
18. Ding Y, Hong J. The signature lncRNAs associated with the lung adenocarcinoma patients prognosis. Math Biosci Eng. 2019;17(2):1593-1603.
19. Jin D, Song Y, Chen Y, Zhang PJ. Identification of Three lncRNAs as Potential Predictive Biomarkers of Lung Adenocarcinoma. Biomed Res Int. 2020;7573689.
20. Wu Y, Lin L, Liu XJDm: Identification of PDL1-Related Biomarkers to Select Lung Adenocarcinoma Patients for PD1/PDL1 Inhibitors. Dis Markers. 2020;2020:7291586.
21. Wang R, Ma ZH, Feng L, Yang YF, Tan C, Shi Q, et al. LncRNA MIR31HG targets HIF1A and P21 to facilitate head and neck cancer cell proliferation and tumorigenesis by promoting cell-cycle progression. Mol Cancer 2018;17(1):162.
22. Yan JL, Dutta B, Hee YT, Chng WJ. Towards understanding of PRC2 binding to RNA. Rna Biol. 2019; 16(2):176-184.
23. Li SQ, Chen Q, Qin HX, Yu YQ, Liao WJ. LINC00152 promotes hepatocellular carcinoma progression by regulating PI3k/Akt/mTOR signaling pathway through miR-139/PIK3CA. Am J Clin Pathol. 190; (5):1095-1107.
24. Bingqing, Hui, Hao, Ji, Yetao, Xu, Juan, Wang, Zhonghua, Death MJC et al: RREB1-induced upregulation of the lncRNA AGAP2-AS1 regulates the proliferation and migration of pancreatic cancer partly through suppressing ANKRD1 and ANGPTL4. Cell Death Dis. 2019;10(3):207.

25. Dong H, Wang W, Mo S, Chen R, Zou K, Jing H, et al. SP1-induced lncRNA AGAP2-AS1 expression promotes chemoresistance of breast cancer by epigenetic regulation of MyD88. J Exp Clin Cancer Res. 2018;37(1):202.
26. Chen J, Peng X, Dai Y. The Long Non-Coding RNA (lncRNA) AGAP2-AS1 is Upregulated in Ovarian Carcinoma and Negatively Regulates lncRNA MEG3. Med Sci Monit. 2019;25:4699-4704.
27. Taniue K, Akimitsu N. The Functions and Unique Features of LncRNAs in Cancer Development and Tumorigenesis. Int J Mol Sci. 2021;22(2):632.
28. Kumar MM, Goyal R. LncRNA as a Therapeutic Target for Angiogenesis. Curr Top Med Chem. 2017;17(15):1750-1757.
29. Liu J, Zhang Q, Ma NJCT. LncRNA GASAL1 Interacts with SRSF1 to regulate trophoblast cell proliferation, Invasion, and Apoptosis Via the mTOR Signaling Pathway. Cell transplant. 2020;29(1): 963689720965182.
30. Tao, Huang, Jian, Wang, Yuan, Zhou, Yi, Zhao, Donghua, Reports HJB: LncRNA CASC2 is upregulated in osteoarthritis and participates in the regulation of IL-17 expression and chondrocyte proliferation and apoptosis. Biosci Rep. 2019;39(5):BSR20182454.
31. Gumiela D. The role of long non-coding RNA (lncRNA) in the development of ovarian cancer. Curr Gynecol Oncol 2020;18 (2): 46–56.
32. Fan Y, Chen M, Zhu QJIA. IncLocPred: Predicting LncRNA Subcellular Localization Using Multiple Sequence Feature Information. 2020;8(99):1-1.
33. Bridges MC, Daulagala AC, Kourtidis A. LNCcation: lncRNA localization and function. J Cell Biol. 2021; 220(2):202009045.
34. Zhang G, Lan Y, Xie A, Shi J, Zhao H, Xu L, et al. Comprehensive analysis of lncRNA-chromatin interactions reveals lncRNA functions dependent on binding diverse regulatory elements. J Biol Chem. 2019;294(43):15613-15622.
35. Li C, Chen J, Zhang K, Feng B, Wang R, Chen LJCP, et al. Biochemistry: Progress and Prospects of Long Noncoding RNAs (lncRNAs) in Hepatocellular Carcinoma. Cell Physiol Biochem. 2015;36(2):423-434.
36. Sauvageau M. DivergingRNPs: Toward Understanding lncRNA-Protein Interactions and Functions. Adv Exp Med Biol. 2019; 1203:285-312.
37. Hochberg-Laufer H, Schwed-Gross A. Uncoupling of nucleo-cytoplasmic RNA export and localization during stress. Nucleic Acids Res. 2019;47(9):4778-4797.
38. Huang Y, Xu Y, Lu Y, Zhu S, Wang ZJB: lncRNA Gm10451 regulates PTIP to facilitate iPSCs-derived β-like cell differentiation by targeting miR-338-3p as a ceRNA. Biomaterials. 2019; 216:119266.
39. Feng T, Zhang Q, Li Q, Zhu T, Qian BJF. Function: LUAD transcriptomic profile analysis of d-limonene and potential lncRNA chemopreventive target. Food Funct. 2020;11(8):7255-7265.
40. Zhao X, Li X, Zhou L, Ni J, Yan W, Ma R, et al. LncRNA HOXA11-AS drives cisplatin-resistance of human LUAD cells via modulating miR-454-3p/Stat3. 2018.
41. Cao H, Gao R, Chen L, Feng YJFOB: TRIM66 promotes malignant progression of prostate carcinoma through the JAK/STAT pathway. FEBS Open Bio. 2020;10(4):515-524.
42. Chen BR, Deshpande A, Barbosa KO, Kleppe M, Deshpande AJB. A Jak/Stat-Mediated Inflammatory Signaling Cascade Drives Oncogenesis in Af10-Rearranged Aml. Blood. 2021;137(24): 3403-3415.
43. Yang F, Zhang S, Meng Q, Zhou F, Yu YJPP, Therapeutics: CXCR1 correlates to poor outcomes of EGFR-TKI against advanced non-small cell lung cancer by activating chemokine and JAK/STAT pathway. Pulm Pharmacol Ther. 2021;67:102001.
44. Yu Z, Wang D, Tang YJM, Biochemistry C: PKM2 promotes cell metastasis and inhibits autophagy via the JAK/STAT3 pathway in hepatocellular carcinoma. Mol Cell Biochem. 2021;476(5): 2001-2010.
45. Ying L, Hl A, Xiang LA, Bei H, Jing X. Molecular insight, expression profile and subcellular localization of two STAT family members, STAT1a and STAT2, from Japanese eel, Anguilla japonica. Gene. 2021;769:145257.